Biol Blood Marrow Transplant 19 (2013) 62–68

French Multicenter 22-Year Experience in Stem Cell Transplantation for Beta-Thalassemia Major: Lessons and Future Directions

Claire Galambrun [1,2,*], Corinne Pondarré [2,3], Yves Bertrand [2,3], Anderson Loundou [4], Pierre Bordigoni [5], Pierre Frange [6], Patrick Lutz [7], Valérie Mialou [2,3], Hervé Rubie [8], Gérard Socié [9], Pascale Schneider [10], Françoise Bernaudin [11], Catherine Paillard [12], Gérard Michel [1,2], Catherine Badens [2,13], Isabelle Thuret [1,2], for the French Rare Disease Center for Thalassemia and the French Society of Bone Marrow Transplantation

[1] *Department of Pediatric Hematology and Oncology, Service d'Hématologie Pédiatrique, Hôpital de la Timone, Marseille, France*
[2] *Rare Disease Center for Thalassemia, Centre de référence maladies rares Thalassémies, Marseille-Lyon, France*
[3] *Institute of Pediatric Hematology and Oncology, Institut d'Hématologie et d'Oncologie Pédiatrique, Lyon, France*
[4] *Unit for Clinical and Epidemiological Research, DRRC/AP-HM Faculté de Médecine de Marseille, Marseiile, France*
[5] *Department of Pediatric Hematology and Oncology, Service d'Hématologie et d'Oncologie Pédiatrique, Hôpital Brabois, Vandoeuvre les Nancy, France*
[6] *Department of Pediatric Hematology and Immunology, Service d'Hématologie et d'Immunologie Pédiatrique, Hôpital Necker, Paris, France*
[7] *Department of Pediatric Hematology and Oncology, Service d'Oncologie et d'Hématologie Pédiatrique, Hôpital Hautepierre, Strasbourg, France*
[8] *Department of Pediatric Hematology and Oncology, Service d'Hématologie et d'Oncologie Pédiatrique, Hôpital des Enfants, Toulouse, France*
[9] *Department of Hematology and Bone Marrow Transplantation, Service d'Hématologie et de greffes de moelle osseuse, Hôpital St Louis, Paris, France*
[10] *Department of Pediatric Hematology and Oncology, Service d'Hématologie et d'Oncologie Pédiatrique, Hôpital Charles Nicolle, Rouen, France*
[11] *Rare Disease Center for Sickle Cell Disease, Centre de référence Drépanocytose, Centre Hospitalier Intercommunal de Créteil, Créteil, France*
[12] *Department of Pediatric Hematology and Oncology, Service d'Hématologie et d'Oncologie Pédiatrique, Hôpital Hôtel Dieu, Clermont-Ferrand, France*
[13] *Molecular Genetics Unit, Department of Genetics, Laboratoire de Génétique Moléculaire, Hôpital d'enfants de la Timone, Marseille, France*

Article history:
Received 27 June 2012
Accepted 7 August 2012

Key Words:
Thalassemia
HSC transplant
Antithymocyte globulin

A B S T R A C T

Although hematopoietic stem cell transplantation (HSCT) offers curative potential for beta-thalassemia major (beta-TM), it is associated with a variable but significant incidence of graft rejection. We studied the French national experience for improvement over time and the potential benefit of antithymocyte globulin (ATG). Between December 1985 and December 2007, 108 patients with beta-TM underwent HSCT in 21 different French transplantation centers. The majority of patients received a matched sibling transplant (n = 96) and a busulfan- and cyclophosphamide-based conditioning regimen (n = 95), also with ATG in 57 cases. Ninety-five of the 108 patients survived, with a median follow-up of 12 years. Probabilities of 15-year survival and thalassemia-free survival after first HSCT were 86.8% and 69.4%, respectively. Graft failure occurred in 24 patients, 11 of whom underwent a second HSCT. The use of ATG was associated with a decrease in rejection rate from 35% to 10%. Thalassemia-free survival improved significantly with time, reaching 83% in the 54 patients undergoing HSCT after 1994 (median time of HSCT). In view of the increased risk of graft rejection after matched sibling HSCT, current French national guidelines recommend, for all children at risk for beta-TM, the systematic addition of ATG to the myeloablative conditioning regimen and special attention to optimize transfusion and chelation therapy in the pretransplantation period.

INTRODUCTION

Beta-thalassemia major (beta-TM) is a genetic disorder resulting in absent or reduced β-globin chain synthesis, causing hemolytic anemia and ineffective erythropoiesis, with massive erythroid hyperplasia in the bone marrow and extramedullary sites. Standard treatment combining life-long RBC transfusions and iron chelation therapy has dramatically improved patient survival over the past 4 decades [1]. Both life expectancy and quality of life with this supportive therapy remain impaired, however. Despite the recent promising results of gene therapy [2], in clinical practice allogeneic hematopoietic stem cell transplantation (HSCT) remains the sole curative option and is largely performed worldwide in patients with an HLA-identical sibling donor.

The Pesaro group in Italy first demonstrated associations between transplantation-related mortality and graft failure

Financial disclosure: See Acknowledgments on page 67.

* Correspondence and reprint requests: Claire Galambrun, Centre de référence des Thalassémies, Service d'Hématologie Pédiatrique, Hôpital d'enfants de la Timone, Marseille, 264 Rue Saint Pierre, 13-385 Marseille Cedex 5, France.
E-mail address: claire.galambrun@ap-hm.fr (C. Galambrun).

1083-8791/$ – see front matter © 2013 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2012.08.005

Table 1
Patient, Donor, and First HSCT Characteristics

Number of patients	108
Sex, n (%)	
Male	56 (52)
Female	52 (48)
Age at transplantation, years, median (range)	6.2 (0.7-32)
≥18 years	11 (10)
≤2 years	15 (14)
Recipient CMV status, n (%)	
Positive	75 (72)
Negative	29
NA	4
Type of donor, n (%)	
MSD	96 (89)
Matched other related	6
URD	6
Stem cell source, n (%)	
Bone marrow	96 (89)
Cord blood	9 (1 combined with BM)
Peripheral blood stem cells	3
Donor age, years, median (range)	9.5 (5-63)
Conditioning regimen, n (%)	
Busulfan + cyclophosphamide	95 (88)
Busulfan + fludarabine, with or without thiotepa	8
Total body irradiation/thoracoabdominal irradiation	5
Use of ATG, n (%)	
Yes	57 (54)
No	49 (46)
NA	2
GVHD prophylaxis, n (%)	
Cyclosporine A	31 (29)
Cyclosporine A + methotrexate	64 (59)
Cyclosporine A + mycophenolate mofetil	1
Cyclosporine A + corticosteroid	9
T cell depletion	3
Nucleated cell dose, $\times 10^8$/kg, median (range)*	3.3 (0.7-11.9)

NA indicates not available.
* For BMT without T cell depletion, data are available for only 72 of 93 patients.

with patient age, degree of iron overload, and liver viral infections [3,4]. The group correlated transplantation outcome with 3 risk categories (Pesaro classes 1-3) based on 3 established severity criteria: hepatomegaly >2 cm, portal fibrosis of any degree, and inadequate compliance with deferoxamine regimen [3,4]. The HCST therapeutic approach pioneered by the Pesaro group is now applied worldwide.

Since the early 1990s, between 130 and 200 HSCTs per year for thalassemia have been registered by the European Group for Blood and Marrow Transplantation [5]. Numerous patient series and national experiences reported outside Italy have confirmed that the majority of children undergoing HSCT from an HLA-identical sibling can be cured of thalassemia [6-12]. Nonetheless, comparatively worse thalassemia-free survival (TFS) was often reported, especially at the start of transplantation programs [6]. Two important factors that might contribute to poorer outcome are inadequate disease treatment before HSCT and unreliable pretransplantation risk status assessment.

Beta-TM is rare in France, most often seen in migrants from south Europe and north Africa. According to the recently established French national thalassemia registry, approximately 350 individuals are currently living with beta-TM in France [13]. A French bone marrow transplantation (BMT) program for patients with beta-TM was initiated soon after the first genoidentical transplantations reported by the Pesaro group. The present study retrospectively analyzed the results of the national BMT program from its inception in 1985 to 2007. A total of 108 consecutive patients with beta-TM who underwent HSCT were included, 90% of whom received an HLA-identical matched sibling donor (MSD) graft. Because we initially and unexpectedly experienced a high rate of graft rejection, we progressively added antithymocyte globulin (ATG) to the myeloablative regimen. Outcomes have improved markedly in recent years, with an 83% TFS rate among the patients undergoing HSCT after 1994 (median date of all transplantations). Based on our evaluation of the benefit of ATG in preventing graft failure, and pending a better understanding of the mechanisms that lead to graft failure in the setting of beta-TM, we believe that ATG should be considered in addition to myeloablative conditioning in these patients.

PATIENTS AND METHODS

Exhaustive identification of patients was attempted through the PROMISE European software, recording BMT data and the French thalassemia registry. Between December 1985 (the date of the first HSCT registered in France) and December 2007, a total of 108 patients with beta-TM major underwent HSCT in 21 different French transplantation centers. Charts of study patients were analyzed retrospectively through 2 professional networks: clinicians of the French Society of Stem Cell Transplantation and Cellular Therapy and the French thalassemia registry. Informed consent was obtained from all patients or their parents for inclusion in both the French registry and the European HSCT database. Data were collected between September 2009 and June 2010, and analysis was performed in September 2010. All survivors had at least 2 years of follow-up after HSCT.

Patient Characteristics

Recipient and donor characteristics are summarized in Table 1. The median age at the time of transplantation was 6.2 years (range, 0.7-32 years). Only 11 patients were adults age >18 years, and 15 were very young children who underwent transplantation at age ≤2 years.

Patients were retrospectively stratified as much as possible according to the Pesaro risk factors: presence of hepatomegaly >2 cm below the costal margin; presence of portal fibrosis on liver biopsy; iron chelation therapy with deferoxamine initiated within 18 months after the first transfusion and administered s.c. at least 5 days/week. However, liver biopsy specimens were obtained in only 42% of the patients, and reliable assessment of the quality of pretransplantation iron chelation was not always available in this retrospective study. Of the 45 recipients who underwent liver biopsy before transplantation, 15 exhibited histological evidence of portal fibrosis. Among the patients who did not undergo liver biopsy, those age <2 years or undergoing transplantation within the year after the first transfusion were considered free of fibrosis.

The patients were assigned into 5 risk categories: class 1 (22 patients), class 1 or 2 (24 patients), class 2 (49 patients), class 2 or 3 (8 patients), and class 3 (5 patients). Twenty-seven patients had undergone splenectomy before transplantation. At the time of transplantation, serum ferritin values were available for 81 patients, ranging from 80 to 5900 ng/mL (median, 1400 ng/mL). At transplantation or/and at the last evaluation, 86 patients were negative and 14 were positive for hepatitis C virus antibodies. Four patients who died before the availability of hepatitis C virus testing were not investigated, and hepatitis C status was unknown for 4 other patients. More than one-half (54%) of the related donors were heterozygous for beta-TM.

Transplantation Procedure

Details on conditioning regimens and graft-versus-host disease (GVHD) prophylaxis are provided in Table 1. All patients received a myeloablative conditioning regimen, most (95 of 108) with oral busulfan (n = 72) or i.v. busulfan (n = 23) combined with cyclophosphamide (200 mg/kg total dose). The remaining 8 patients, all treated after 2004, received fludarabine, 7 in combination with busulfan and thiotepa. In the first years of the program, 5 patients received conditioning including irradiation (5 or 6 cGy thoracoabdominal irradiation or total body irradiation). Owing to the numerous rejections occurring in recipients of first HSCT, ATG was introduced and used increasingly over time in association with busulfan and cyclophosphamide to facilitate engraftment. Fifty-seven patients received ATG as part of conditioning. Before April 1994 (the median date of HSCT in this study), only 17 of 54 patients (31%) received ATG, compared with 40 of 54 (74%) after 1994. ATG was used systematically in all 6 patients receiving an unrelated donor (URD) transplant.

The stem cell source was bone marrow in 96 patients, peripheral blood stem cells in 3 patients, cord blood in 8 patients, and combined cord blood

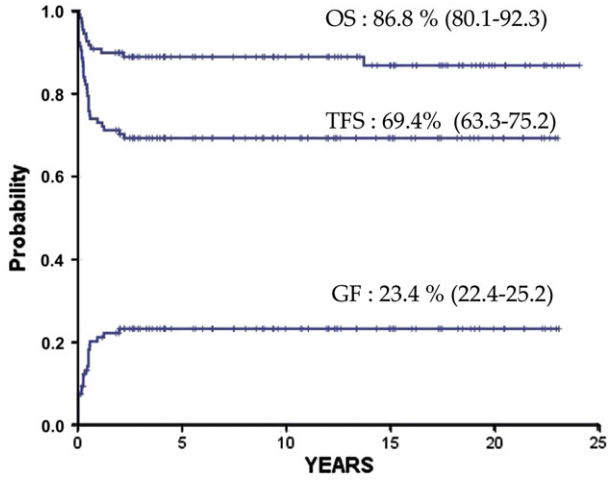

Figure 1. Actuarial 15-year probabilities of OS, TFS, and graft failure after first HSCT in all patients.

and bone marrow from the same sibling donor in 1 patient. The majority of transplants (96 of 108) and all cord blood transplants (9 of 9) were from an HLA-MSD. Six patients received a transplant from a phenotypic HLA-identical parent or relative, and 6 others received a transplant from a matched URD.

In the majority of patients (88%), GVHD prophylaxis consisted of cyclosporine A alone (31 patients) or a combination of cyclosporine A and methotrexate (64 patients). In 7 of 9 cord blood recipients, GVHD prophylaxis included only cyclosporine A, without methotrexate. Supportive therapy and posttransplantation use of hematopoietic growth factors were in accordance with the policy of each individual center.

Definition of Endpoints

The primary study endpoints were TFS, graft failure, and overall survival (OS). TFS was defined as survival without graft failure or a second transplantation. Graft failure was clinically defined as persistent pancytopenia with no hematological recovery or recurrent thalassemia with relapse of transfusion dependence. Chimerism data were incomplete and when available were captured by different techniques over time (eg, globin chain synthesis, YO chromosome hybridization, analysis of variable number tandem repeat polymorphisms, microsatellite analysis). For analysis of OS, failure was defined as death from any cause, and surviving patients were censored at the date of last contact.

Statistical Analyses

Percentages derived from univariate analysis were compared using the χ^2 test or Fisher exact test for dichotomous variables and Pearson's exact χ^2 test for qualitative variables of more than 2 categories. For continuous variables, medians were calculated and compared using the nonparametric Mann-Whitney *U* test.

Univariate probabilities of OS and TFS were calculated using the Kaplan-Meier estimator, and their 95% confidence intervals (CIs) were constructed using arcsine-transformed intervals. For actuarial probability of graft failure, the 95% CI of a cumulative terminating event was constructed using log-log approach. The log-rank test was used to compare survival probabilities.

The impact of the following variables on TFS, graft failure, and OS were examined: (1) recipient-related factors (age, sex, and pretransplantation CMV status); (2) disease-related factors (Pesaro risk class, splenectomy); (3) donor-related factors (sex mismatch, thalassemia status); and (4) transplantation-related factors (graft source [bone marrow, cord blood, or peripheral blood stem cells], type of transplant [MSD or others], type of conditioning [busulfan + cyclophosphamide, irradiation, fludarabine], use of ATG, and year of transplantation [before or after April 1994, the median date of HSCT in this study]).

Multivariate Cox proportional hazards regression was performed for the variables that were identified as associated with one of the endpoints or were marginally significant ($P < .10$) on univariate analysis, or that had clinical relevance (ie, age). Logistic regression was used for multivariate analysis of graft failure.

For all tests, *P* values were 2-sided, and statistical significance was defined as $P < .05$. All analyses were done with PASW Statistics version 17.0 (SPSS, Inc., Chicago, IL).

Table 2
Univariate Analysis of TFS after First Transplantation

	Number of Events	% (95% CI)	*P* Value
Year of transplantation			.001
1985-1994	54	55.6 (48.1-62.9)	
1994-2007	54	83.3 (74.1-90.5)	
Age at transplantation			.266
≤2 years	15	53.3 (39.9-66.5)	
2-8 years	82	73.2 (65.8-79.8)	
≥18 years	11	63.6 (45-80.3)	
Splenectomy			.089
Yes	27	85.2 (72.3-94.6)	
No	78	66.7 (59.3-73.3)	
Type of transplant			.003
MSD	96	74.0 (67.1-80.1)	
Other	12	33.3 (24.8-42.4)	
Donor thalassemia status			.40
Heterozygote	51	74.5 (65.1-82.8)	
Thalassemia-free	44	68.2 (58.2-77)	
Recipient CMV status			.70
Positive	75	69.3 (61.9-76.2)	
Negative	29	72.4 (60-83.3)	
Pesaro class			<.001
1	22	81.8 (67.1-92.9)	
1 or 2	24	75 (60.5-86.3)	
2	49	73.5 (64-82)	
2 or 3	8	25.0 (17.9-32.8)	
3	5	20.0 (13.5-27.4)	
Use of ATG			.002
Yes	57	82.5 (9.9)	
No	49	55.1 (13.9)	

RESULTS

OS, TFS, and Status at Last Follow-Up

Actuarial 15-year OS and TFS were 86.8% (95% CI, 80.1%-92.3%) and 69.4% (95% CI, 63.3%-75.2%), respectively (Figure 1). Twenty-four patients experienced graft failure after first HSCT, and 13 patients died. The primary causes of death were GVHD (n = 4), multiorgan failure (n = 2), CMV pneumonitis (n = 2), neurotoxicity (n = 1), gram-negative sepsis in a splenectomized patient (n = 1), secondary hemochromatosis after graft failure (n = 1), and primary graft failure after first allograft (n = 1) or second allograft (n = 1). All deaths occurred during the first 2 years posttransplantation except for 1 death due to cardiac iron overload occurring 13.7 years after graft failure.

At the time of this report, of the remaining 95 patients, with a median follow-up duration of 12 years, 81 are alive and free of thalassemia (75 after first HSCT, 6 after second HSCT) and 14 have transfusion-dependent anemia. Eight patients (3 patients with graft failure and 5 successfully transplanted) were lost to follow-up after at least 2 years posttransplantation. The overall proportion of patients with TFS increased to 75% when second HSCTs were taken into account.

The results of univariate and multivariate analyses are presented in Tables 2-5. Pesaro risk classification, despite its approximate use in our report, had a significant association with OS and TFS in both univariate and multivariate analyses. On univariate analysis, the OS was significantly worse for adults (63.6% for the 11 adult patients).

Type of donor had a significant association with TFS, greater for recipients of an MSD graft (74%) than for recipients of other donor grafts (33%), with 3 of 6 patients and 1 of 6 patients alive without thalassemia after a URD transplant and a familial matched related (other than MSD) transplant, respectively. The 9 patients who received a cord blood transplant, all from an MSD, are alive and cured. Stem cell

Table 3
Univariate Analysis of Graft Failure after First Transplantation

	Number of Events	% (95% CI)*	*P* Value
Year of transplantation			<.001
1985-1994	20	37.0 (24.2-49.8)	
1994-2007	4	7.4 (0.5-13.3)	
Age at transplantation			.35
≤2 years	5	33.3 (9.5-57.1)	
2-8 years	18	22 (13.1-30.9)	
≥18 years	1	9.1 (0.5-43)	
Splenectomy			.09
Yes	2	7.4 (1.3-25.8)	
No	19	24.4 (14.9-33.9)	
Type of transplant			.134
MSD	19	19.8 (11.9-27.7)	
Other	5	41.7 (13.9-69.5)	
Donor thalassemia status			.38
Heterozygote	9	17.6 (7.2-28)	
Thalassemia-free	11	25 (12.8-37.2)	
Recipient CMV status			.027
Positive	20	26.7 (16.7-36.7)	
Negative	2	6.9 (1.2-24)	
Pesaro class			.326
1	3	13.6 (3.6-40)	
1 or 2	6	25 (7.7-42.3)	
2	10	20.4 (9.2-31.6)	
2 or 3	4	50 (17.5-82.6)	
3	1	20 (1.1-70)	
Use of ATG			.003
Yes	6	10.5 (2.6-18.4)	
No	17	34.7 (21.4-48)	

* 95% CI including continuity correction for small numbers.

source had no statistically significant impact on TFS or graft rejection, likely because of the low number of patients receiving cord blood transplantation.

The use of ATG and more recent HSCT were significantly associated with better TFS on univariate analysis (TFS of 55% and 83%, respectively) (Figure 2A and B). These 2 variables were related as ATG use increased over time; 31% of patients who underwent HSCT before April 1994 received ATG, versus 74% of those who did so after this date. Only the year of HSCT remained significant in multivariate analysis.

Splenectomy was an independent factor associated with better TFS. Although older, with a median age of 11.2 years, the 27 splenectomized patients did not have more advanced disease or higher serum ferritin levels (Table 6). Of note, none of the 15 patients age ≤2 years was splenectomized, and TFS was poor in this age group (5 graft failures and 2 deaths).

Table 4
Multivariate Cox Regression Analysis of TFS after First Transplantation

	Relative Risk	95% CI	*P* Value
Year of transplantation			
1985-1994	1		
1994-2007	0.32	0.11-0.93	.036
Age at transplantation	1.02	0.94-1.11	.609
Type of transplant			
MSD	1		
Other	4.21	1.54-11.6	.005
Use of ATG			
No	1		
Yes	0.5	0.10-1.31	.162
Splenectomy			
No	1		
Yes	0.12	0.03-0.57	.007
Pesaro class			.004
1	1		
1 or 2	1.27	0.35-4.57	.72
2	1.19	0.35-4.05	.78
2 or 3	18.28	3.11-107	.001
3	4.46	0.65-30.4	.127

Graft Failure and Second Transplantation

Graft failure after the first transplantation (primary graft failure in 8 cases and rejection or secondary thalassemia in 16 cases) was observed in 24 patients, corresponding to a 15-year probability of 23.4% (95% CI, 22.4%-25.2%). Eleven patients underwent a second allogeneic transplantation: 6 were successfully treated, 2 died, and 3 remained transfusion-dependent. The median interval between the first HSCT and second HSCT was 2 years (range, 1 month to 11 years).

On univariate analysis, a significant difference in the risk of graft failure was observed according to date of transplantation, use of ATG, and recipient CMV status. Splenectomy before transplantation was nearly statistically significant ($P = .07$). On multivariate analysis, only recipient CMV status retained statistical significance. Donor thalassemia status, recipient sex, donor–recipient sex mismatch, stem cell source, and type of conditioning had no significant impact on any of the 3 primary study endpoints.

GVHD and Veno-Occlusive Disease

Grade II-IV acute GVHD occurred in 22 patients and was lethal in 2 patients, both of whom received an MSD transplant. Chronic GVHD occurred in 12 patients (limited in 6, extensive in 6, and lethal in 2, [1 after an URD HSCT and the 1 after a matched related donor HSCT]). The use of ATG had no apparent association with the risk of acute GVHD (data not shown). Fourteen patients developed hepatic veno-occlusive disease after first HSCT, but only 1 patient died of this complication.

DISCUSSION

In this study, we retrospectively analyzed all patients who underwent HSCT for beta-TM in France between December 1985 (when the first transplantation was registered) and December 2007. This national multicenter study was exhaustive and reflected the global results of this treatment modality in France. Our median follow-up of 12 years (range, 2-21 years) is one of the longest reported to date. HSCT was performed quite early in childhood, with a median age at transplantation of 6.2 years for the 108 patients, but was rarely proposed to adults, with only 11 undergoing HSCT. Transplant procedures were generally standard, with the vast majority of patients receiving bone marrow from an HLA-identical MSD and a myeloablative conditioning regimen including busulfan and cyclophosphamide. Given that only 350 persons with beta-TM (including HSCT recipients) are listed in the French thalassemia registry, allogeneic HSCT has been considered as an option for most children with an MSD [13].

In contrast, URD HSCT was rarely performed and was associated with poor outcome. Although donor selection based on stringent compatibility criteria has increased the probability of TFS in some studies, URD HSCT is associated with significant risk of GVHD [14]. Thus, French transplantation guidelines for thalassemia call for consideration of URD HSCT only in extreme situations, such as alloimmunization precluding transfusion.

Considering the large number of involved transplantation centers (n = 21) and the long observation time (more than 20 years), patient survival was good. Actuarial 15-year OS was 86.8% with a median follow-up of 12 years in survivors.

Table 5
Logistic Regression Analysis of Graft Failure after First Transplantation

	Odds Ratio	95% CI	*P* Value
Year of transplantation			
1985-1994	1		
1994-2007	0.21	0.04-1.19	.078
Age at transplantation	0.84	0.68-1.04	.098
Type of transplant			
MSD	1		
Other	2.44	0.34-17.7	.38
Use of ATG			
No	1		
Yes	0.19	0.03-1.14	.07
Splenectomy			
No	1		
Yes	0.14	0.01-1.35	.089
Pesaro class		0.40-30.2	.06
Recipient CMV status			
Negative	1		
Positive	17.23	2.42-122	.004

The main complication encountered was graft failure, which occurred in 23% of cases. The incidence of graft failure was particularly high in HSCTs performed before 1994, reaching 38%. As a result, intensified immunosuppression with the addition of ATG to pretransplantation myeloablative conditioning regimens became increasingly popular over time. In HSCTs performed after 1994, with the more frequent use of ATG, the incidence of graft failure decreased to 7% and TFS improved, reaching similar values as those recently reported in Italy and elsewhere (Figure 2A and B) [11,15,16]. Surprisingly, even in the Pesaro class 1 or 2 low transplantation risk group, without ATG, our results were far worse (graft failure rate of 29%; 11 of 38 patients) than those obtained by the Italian group, who reported a rejection rate of only 8% after MSD HSCT during the same time period [4]. Interestingly, in our experience, with the addition of ATG, graft failure rate decreased significantly to 7% in these low-risk patients (3 of 46 patients; $P = .012$). Examination of the role of ATG in reducing graft failure is limited by the global improvement in supportive care over the study period, as well as other changes in transplantation procedures, such as the introduction of i.v. busulfan and pharmacokinetic busulfan monitoring. Because the present study was retrospective and involved a large number of participating centers, we do not know precisely how often busulfan levels were monitored and cannot determine the impact of pharmacokinetic monitoring on transplantation endpoints.

According to the Italian group, the addition of ATG is recommended only for high-risk transplantations (class 3 children, adults, URD HSCT, or second HSCT) and is not required for class 1 or 2 recipients of MSD HSCT [14,17-20]. We are not the first group to report such a high incidence of graft failure, however, even in the low transplant risk group. Outside Italy, numerous studies have investigated the use of immunosuppressive agents, including ATG and Campath1G, in addition to the busulfan/cyclophosphamide conditioning regimen to reduce the incidence of graft rejection [6,7,9,11]. Interestingly, for sickle cell disease, another hemoglobinopathy, the use of ATG has proven successful in reducing graft rejection, and the most experienced French group now recommends ATG in addition to myeloablative conditioning [21].

A possible explanation for the high rejection rate observed in the present study is suboptimal medical care of this rare disease before HSCT. Obviously, pretransplantation evaluation was insufficient, given that few patients were

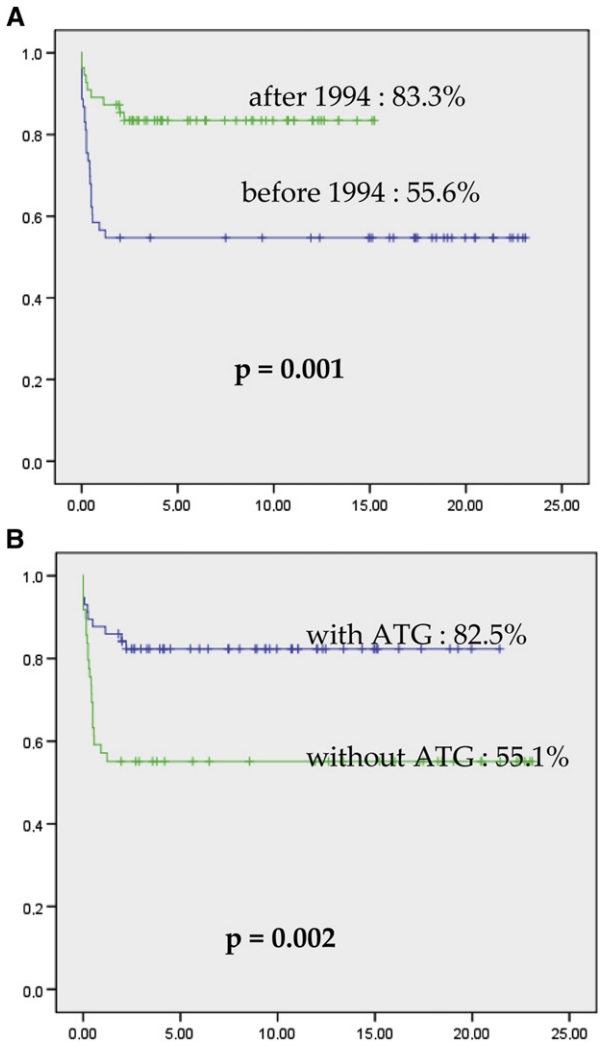

Figure 2. Comparative actuarial 15-year probability of TFS after first HSCT.

accurately assigned to 1 of the 3 Pesaro risk categories. Given the missing data and lack of systematic liver histological evaluation, we applied an approximate stratification. Of note, the value of the Pesaro classification is well illustrated in our experience. Despite an approximate use, the classification remained significantly associated with transplantation outcomes, illustrating the critical importance of quality medical care before HSCT.

Expanded erythropoietic marrow and splenomegaly have been invoked to explain the increased rate of rejection/disease recurrence in patients with beta-TM. Preconditioning implementation of the transfusion regimen to ablate expanding thalassemic marrow might be a key factor in reducing the risk of graft rejection. Whereas pretransfusion hemoglobin levels are usually maintained at >9-10 g/dL in experimented Italian centers, the transfusion schemes used in France are more variable, with baseline hemoglobin values often below 9 g/dL [22-24]. Because these levels have been associated with insufficient inhibition of erythroid marrow expansion, we believe that suboptimal transfusion might have contributed to the high rejection rate initially observed in the French experience [23]. Current French guidelines use a transfusion scheme based on the maintenance of minimum pretransfusion hemoglobin >10 g/dL starting at 3-6 months before conditioning [25].

Table 6
Baseline Characteristics in Patients with and without Pretransplantation Splenectomy

	Splenectomy (n = 27)*	No Splenectomy (n = 78)*	*P* Value
Median ferritin level, ng/mL	925	1546	.269
Median age, years	11.2	4.9	<.001
Age ≤2 years, n	0	15	.02
Median nucleated cell dose, × 10^8/kg	3.26	3.3	NS
Pesaro class 2-3 or 3, n	4/27	7/78	NS
HSCT before 1994, n	14/27	37/78	NS
Use of ATG, n	16/27	40/77	NS
Recipient CMV^+ status, n	21/27	51/74	NS

NS indicates not significant.
* Data on splenectomy were not available for 3 patients.

In this national experience, 4 prognosis factors for TFS were identified: use of an MSD, year of HSCT, Pesaro classification, and splenectomy (Tables 3 and 4). Surprisingly, the observed effect of splenectomy was independent. Although older (median age, 11.2 years), the 27 splenectomized patients had a better TFS than the nonsplenectomized patients. A possible explanation for this finding is that splenectomy reflects better medical care of the disease before HSCT rather than an advanced stage of the disease. Indeed, there were no more class 2 or 3 patients in the group of splenectomized patients, and their median serum ferritin value was <1000 ng/mL. In one study analyzing the impact of splenectomy on transplantation outcomes, patients who underwent splenctomy before transplantation exhibited faster neutrophil recovery, suggesting that splenomegaly could have a potential adverse impact on engraftment, through sequestration of infused cells [26]. Although the benefit of pretransplantation splenectomy remains unclear, the increased risk of infection associated with this procedure argues against its use. Finally, in the present study, very young age was associated with a TFS of only 53%; 7 of the 15 patients age ≤2 years experienced graft rejection (n = 5) or died (n = 2). No infants were splenectomized. Our experience is not isolated, however; Lucarelli et al. [27] also reported poorer outcomes in young children. Consequently, since 2003, the Italian group has applied more intensive conditioning (thiotepa 10 mg/kg/day in addition to the standard busulfan + cyclophosphamide) for children age <4 years [27]. The reason for this increased rejection rate is unclear, but it might be explained by the greater variability in busulfan kinetics in low-weight infants, even with i.v. formulations or by undertransfusion. Our results suggest that delaying HSCT until the child is at least 2 years old is a reasonable approach, to allow efficient suppression of thalassemic hematopoiesis by regular transfusion and avoid poor busulfan kinetics.

Considering rejection, only recipient CMV^+ status was independently associated with a higher rate of graft failure ($P = .004$; Tables 4 and 5), with ATG not a significantly independent risk factor for rejection ($P = .07$). Previous data have shown that CMV inhibits the engraftment of the transplanted hematopoietic cells and can cause complete graft failure. CMV infection itself as well as its antiviral treatment may jeopardize engraftment and interfere directly with the therapeutic aim of HSCT [28]. In the present cohort study, 2 deaths were attributed to CMV infection, but because of the retrospective nature of this analysis and the long observation time, evaluating the incidence of CMV reactivation was not possible. A recent study of 75 thalassemic children who underwent HSCT with ATG as part of the myeloablative conditioning regimen reported a 29% rate of CMV reactivation, but no CMV disease [12]. Interestingly, this high rate of CMV reactivation did not jeopardize the success of bone marrow engraftment; both transplantation-related mortality and graft failure rate were only 4% for the entire cohort.

In the present study, 22 patients developed grade II-IV acute GVHD, an incidence rate similar to that reported by others [29]. The rate of infectious complications, including bacterial sepsis and CMV disease, was low, as was the rate of VOD.

Several recent changes in transplantation procedures may further improve outcomes in patients with beta-TM [30-35]. Because our analysis stopped in December 2007, most of our patients had received oral busulfan, which has since been replaced by i.v. busulfan with drug monitoring, allowing dosage adjustment and limiting toxicity [33-36]. In our experience, as in a recent study, related cord blood transplantation was associated with very good outcomes. Because of more systematic cord blood preservation in families with a thalassemic child, related cord blood transplantation is expected to become increasingly popular [15,30].

These national results confirm that MSD HSCT is an effective therapeutic option for beta-TM. Current French national guidelines recommend HLA-matched identical sibling HSCT early in the course of the disease and consider myeloablative conditioning including ATG as a standard of care, even for low-risk patients. We believe that special attention should be paid to quality of care before transplantation, and that optimization of both the chelation regimen and the transfusion program to sufficiently suppress erythropoiesis is crucial before conditioning. The decision to recommend ATG in addition to a myeloablative regimen was based on the balance between the increased risk of infection associated with intensified immunosuppression and the greater risk of marrow rejection without ATG. In hemoglobinopathies, recent studies including ATG as part of the preparative regimen and systematic monitoring for viral infection to allow preemptive treatment seem to provide excellent results [12,21]. With the dissemination of the recent French national guidelines for transplantation in patients with beta-TM, better knowledge of this rare disease by hematology and transplant teams in our country should contribute to improved disease evaluation and patient preparation before transplantation.

ACKNOWLEDGMENTS

We thank Christelle Grangier, Rare disease center for Thalassemia, for data collection and recording. We also thank the clinicians participating in the registry and the patients and their families for their cooperation.

Authorship Statement: Claire Galambrun, Corinne Pondarré and Isabelle Thuret were the principal investigators, designed the study, took primary responsibility for and wrote the paper. Isabelle Thuret collected and analyzed the data. Catherine Badens and Isabelle Thuret provided administrative support for the French national thalassemia registry. Anderson Loundou performed the statistical analysis. All authors contributed to the critical revision and final approval of the manuscript.

Financial disclosure: The Rare disease center for Thalassemia is supported by The French Ministry of Health. The national thalassemia registry receives support from the Institut National de la Santé et la Recherche Médicale and the

Institut National de Veille Sanitaire. The authors have no conflicts of interest to disclose.

REFERENCES

1. Borgna-Pignatti C, Rugolotto S, De Stefano P, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. *Haematologica*. 2004;89:1187-1193.
2. Cavazzana-Calvo M, Payen E, Negre O, et al. Transfusion independence and HMGA2 activation after gene therapy of human β-thalassemia. *Nature*. 2010;467:318-322.
3. Lucarelli G, Galimberti M, Polchi P, et al. Bone marrow transplantation in patients with thalassemia. *N Engl J Med*. 1990;322:417-421.
4. Giardini C, Angelucci E, Lucarelli G, et al. Bone marrow transplantation for thalassemia: experience in Pesaro, Italy. *Am J Pediatr Hematol Oncol*. 1994;16:6-10.
5. Angelucci E, Baronciani D. Allogeneic stem cell transplantation for thalassemia major. *Haematologica*. 2008;93:1780-1781.
6. Ball LM, Lankester AC, Giordano PC, et al. Paediatric allogeneic bone marrow transplantation for homozygous beta-thalassaemia, the Dutch experience. *Bone Marrow Transplant*. 2003;31:1081-1087.
7. Lawson SE, Roberts IAG, Amrolia P, et al. Bone marrow transplantation for beta-thalassaemia major: the UK experience in two paediatric centres. *Br J Haematol*. 2003;120:289-295.
8. Hongeng S, Pakakasama S, Chuansumrit A, et al. Outcomes of transplantation with related and unrelated donor stem cells in children with severe thalassemia. *Biol Blood Marrow Transplant*. 2006;12:683-687.
9. Ramzi M, Nourani H, Zakerinia M, et al. Results of hematopoietic stem cell transplant in Shiraz: 15 years' experience in southern Iran. *Exp Clin Transplant*. 2010;8:61-65.
10. Fang JP, Xu LH. Hematopoietic stem cell transplantation for children with thalassemia major in China. *Pediatr Blood Cancer*. 2010;55:1062-1065.
11. Sabloff M, Chandy M, Wang Z, et al. HLA-matched sibling bone marrow transplantation for thalassemia major. *Blood*. 2011;117:1745-1750.
12. Goussetis E, Peristeri I, Kitra V, et al. HLA-matched sibling stem cell transplantation in children with beta-thalassemia with anti-thymocyte globulin as part of the preparative regimen: the Greek experience. *Bone Marrow Transplant*. 2012;47:1061-1066.
13. Thuret I, Pondarre C, Loundou A, et al. Complications of patients with β-thalassemia in France: results of the National Registry. *Haematologica*. 2010;95:724-729.
14. La Nasa G, Argiolu F, Giardini C, et al. Unrelated bone marrow transplantation for beta-thalassemia patients: the experience of Italian Bone Marrow Transplant Group. *Ann NY Acad Sci*. 2005;1054:186-195.
15. Lucarelli G, Gaziev J. Advances in the allogeneic transplantation for thalassemia. *Blood Rev*. 2008;22:53-63.
16. Lisini D, Zecca M, Giorgiani G, et al. Donor/recipient mixed chimerism does not predict graft failure with β-thalassemia given an allogeneic cord blood transplant from an HLA-identical sibling. *Haematologica*. 2008;93:1859-1867.
17. Lucarelli G, Clift RA, Galimberti M, et al. Marrow transplantation for patients with thalassemia: results in class 3 patients. *Blood*. 1996;87:2082-2088.
18. Sodani P, Gaziev D, Polchi P, et al. New approach for bone marrow transplantation in patients with class 3 thalassemia aged younger than 17 years. *Blood*. 2004;104:1201-1203.
19. Gaziev J, Sodani P, Lucarelli G, et al. Second hematopoietic SCT in patients with thalassemia recurrence following rejection of the first graft. *Bone Marrow Transplant*. 2008;42:397-404.
20. Lucarelli G, Clift RA, Galimberti M, et al. Bone marrow transplantation in adult thalassemic patients. *Blood*. 1999;93:1164-1167.
21. Bernaudin F, Socié G, Kuentz M, et al., for the SFGM-TC. Long-term results of related myeloablative stem cell transplantation to cure sickle cell disease. *Blood*. 2007;110:2749-2756.
22. Cazzola M, De Stefano P, Ponchio L, et al. Relationship between transfusion regimen and suppression of erythropoiesis in β-thalassemia major. *Br J Haematol*. 1995;89:473-478.
23. Cazzola M, Borgna-Pignatti C, Locatelli F, et al. A moderate transfusion regimen may reduce iron loading in beta-thalassemia major without producing excessive expansion of erythropoiesis. *Transfusion*. 1997;37:135-140.
24. Olivieri NF. The beta-thalassemias. *N Engl J Med*. 1999;341:99-109.
25. Pondarre C, Thuret I, Galambrun C, et al. Recommandations pour les greffes de cellules souches hématopoïétiques dans les bêta-thalassémies. *Correspond Onco-Hématol*. 2012;7:45-50.
26. Mathews V, George B, Lakshmi KM, et al. Impact of pretransplant splenectomy on patients with β-thalassemia major undergoing a matched-related allogeneic stem cell transplantation. *Pediatr Transplant*. 2009;103:171-176.
27. Isgro A, Gaziev J, Sodani P, et al. Progress in hematopoietic stem cell transplantation as allogeneic cellular gene therapy in thalassemia. *Ann NY Acad Sci*. 2010;1202:149-154.
28. Steffens HP, Podlech J, Kurz S, et al. Cytomegalovirus inhibits the engraftment of donor bone marrow cells by downregulation of hematopoietic gene expression in recipient stroma. *J Virol*. 1998;72:5006-5015.
29. Gaziev D, Polchi P, Galimberti M, et al. Graft-versus-host disease after bone marrow transplantation for thalassemia: an analysis of incidence and risk factors. *Transplantation*. 1997;63:854-860.
30. Locatelli F, Rocha V, Reed W, et al. Related umbilical cord blood transplantation in patients with thalassemia and sickle cell disease. *Blood*. 2003;101:2137-2143.
31. Pinto FO, Roberts I. Cord blood stem cell transplantation for haemoglobinopathies. *Br J Haematol*. 2008;141:309-324.
32. Boncimino A, Berating A, Locatelli F. Cord blood transplantation in patients with hemoglobinopathies. *Transfus Apheresis Sci*. 2010;42:277-281.
33. Vassal G, Michel G, Esperou H, et al. Prospective validation of novel IV busulfan fixed dosing for pediatric patients to improve therapeutic AUC targeting without drug monitoring. *Cancer Chemother Pharmacol*. 2008;61:113-123.
34. Gaziev J, Nguyen L, Puozzo C, et al. Novel pharmacokinetic behaviour of intravenous busulfan in children with thalassemia undergoing hematopoietic stem cell transplantation: a prospective evaluation of pharmacokinetic and pharmacodynamic profile with therapeutic drug monitoring. *Blood*. 2010;115:4597-4604.
35. Bernardo ME, Zecca M, Piras E, et al. Treosulfan-based conditioning regimen for allogeneic haematopoietic stem cell transplantation in patients with thalassaemia major. *Br J Haematol*. 2008;143:548-551.
36. Cappelli B, Chiesa R, Evangelio C, et al. Absence of VOD in paediatric thalassaemic HSCT recipients using defibrotide prophylaxis and intravenous Busulphan. *Br J Haematol*. 2009;147:554-560.

APPENDIX

In addition to the authors, the following participating physicians from the French transplantation centers and national thalassemia registry contributed to this study (listed in alphabetic order):

Michel Attal, Hôpital Purpan, Toulouse
Didier Blaise, Hôpital Paoli Calmette, Marseille
Nicolas Blin, Hôpital l'Hôtel-Dieu, Nantes
Bénédicte Bruno, Hôpital Jeanne de Flandre, Lille
Jacques Camerlo, Hôpital Paoli Calmette, Marseille
Catherine Cordonnier, Hôpital Henri Mondor, Créteil
Jean-Hugues Dalle, Hôpital Robert Debré, Paris
Marianne Debré, Hôpital Necker Enfants Malades, Paris
Eric Deconinck, Hôpital Jean Minjoz, Besançon
Stéphane Ducassou, Hôpital Hautepierre, Strasbourg
Gisèle Elana, Hôpital du Lamentin, Martinique
Alain Fischer, Hôpital Necker Enfants Malades, Paris
Eliane Gluckman, Hôpital St. Louis, Paris
Jean-Pierre Jouet, Hôpital Claude Huriez, Lille
Anne Lambliotte, Hôpital Jeanne de Flandre, Lille
Bruno Lioure, Hôpital Hautepierre, Strasbourg
Agathe Masseau, Hôpital l'hôtel-Dieu, Nantes
Mauricette Michalet, Hôpital Edouard Herriot, Lyon
Bénédicte Neveu, Hôpital Necker Enfants Malades, Paris
Yves Perel, Hôpital Pellegrin, Bordeaux
Christophe Piguet, Hôpital Mère-Enfant, Limoges
Emmanuel Plouvier, Hôpital St. Jacques, Besançon
Maryline Poirée, Hôpital Archet, Nice
Antoine Ribeil, Hôpital Necker Enfants Malades, Paris
Marie Robin, Hôpital St. Louis, Paris
Vanderson Rocha, Hôpital St. Louis, Paris
Pierre Rohrlich, Hôpital Jean-Minjoz, Besançon
Alexandra Salmon, Hôpital Brabois, Nancy
Laure Saumet, Hôpital Villeneuve, Montpellier
Jean-Louis Stephan, Hôpital Nord St. Etienne, St. Etienne
Felipe Suarez, Hôpital Necker Enfants Malades, Paris
Caroline Thomas, Hôpital l'hôtel-Dieu, Nantes
Agha Ibrahim Yakoub, Hôpital Claude Huriez, Lille
Karima Yakouben, Hôpital Robert Debré, Paris